REMARKS

Claims 1, 3-32, 34-47, 59, 61-64 and 144-147, which are set forth below, are pending in this application. Claims 16-25, 36-40 and 44-46 are objected to as being dependent on a rejected base claim but would be allowable if rewritten in independent form. In addition, claim 15 is not rejected on any ground.

Claim 11, 26, 28 and 34 are amended for clarity; the amendments do not change the scope of the claims nor are they designed to avoid any art of record. Claims 1 and 34 are amended to render it clear that the a delivery agent is added to a composition, such as cell in tissue culture medium so that the claims cannot read on a method in which nucleic acid molecules are treated with one delivery agent and added to cells in tissue culture medium. Claim 1 is amended by incorporation of the limitation of claim 2, mended to render the meaning of "enhances" and "increases" clear. The specification is very clear that claimed methods require an affirmative step of treating the cells with a delivery agent and treating the nucleic acid with a delivery agent. Claim 26 is rewritten as an independent claim to avoid improperly depending on claim 1. Claim 11 is amended to properly depend from claim 1; and claim 28 is amended to correct an inadvertent and obvious typographical error. Claim 15, which is not rejected on any ground, is rewritten as an independent claim. Therefore, no new matter is added.

THE REJECTION OF CLAIMS 2, 11 AND 26-29 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 2, 11 and 26-29 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the reasons enumerated below. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Claim 2, and claim 11, are rejected as indefinite in the recitation of "increases" and "enhances" because these terms are relative. It is clear from the context of the claim what is meant. In the interest of advancing prosecution, the claims are amended to recite that an agent enhances permeability or increases contact relative to in its absence. Claim 11 as pending was outside the purview of this rejection. Claim 11, however, is amended to depend on claim 2. In also now incorporates the amendments of claims 2, thereby obviating any of these grounds for rejection.

Claims 26 and dependent claims are rejected as being improperly dependent on claim 1. Claim 26 is rewritten as an independent claim, including all prior elements, thereby

obviating this rejection.. can be amended to properly depend from claim 1 by including a recitation of steps (a) and (b) from claim 1 with each of the steps (a) and (b) as pending as a wherein clause from the respective steps. If necessary or expedient it can be rewritten as an independent claim. Claim 28 is rejected as indefinite in failing to reference proper antecedent for "the energy is ultrasound." Amendment of the claim to depend from claim 11 will obviate this rejection.

THE REJECTION OF CLAIMS 1-4, 6, 7, 9, 10, 12-14, 30-32 34, 35, 41-43 AND 47 UNDER 35 U.S.C. §102(b)

Claims 34, 35, 41-43 and 47

Claims 34, 35, 41-43 and 47 are rejected under 35 U.S.C. §102(b) as being anticipated by Unger *et al.*, ((1997) Invest. Radiol. 32:723-727) as evidenced by Mediatech, Inc, which sets for the a table of formulations for Dulbecco's modified Eagle's Medium. The Office Action urges that Unger *et al.*. discloses a method for introducing a nucleic acid molecule into a cell by applying ultrasound in the absence of the nucleic acid molecule and then contacting the cell with the nucleic acid. The Examiner then urges that the medium in which the cells are contacted serves as a delivery agent since it "facilitates delivery into cells." In addition, as evidenced by Mediatech, Inc., the medium contains cationic compounds. This rejection is respectfully traversed.

This rejection as well as the other rejections over art are premised on allegation that providing cells in cell culture medium constitutes "exposing" cells to a delivery agent because cell culture medium contains cationic compounds and other/or other compounds that are delivery agents. Applicant respectfully disagrees with this premise and urges that cell culture medium is not a delivery agent. Further providing cells in medium for mixing the cells with a delivery agent does not constitute adding a delivery agent to a composition containing cells. It is respectfully submitted that this premise is unsupported by any evidence of record and constitutes a strained reading of the claims that is not contemplated by applicant nor that would be so-understood by the ordinarily skilled artisan.

Relevant law

Reference is made to previous responses, which set forth relevant case law that provides the framework for the analysis. The rendition of the relevant case law for anticipation from previous responses in connection with this application is incorporated herein by reference.

In addition, an anticipatory publication must describe the claimed invention with sufficient clarity and specificity so that one skilled in the relevant art could practice the subject matter of the patent without assistance from the patent claimed to have been anticipated Columbia Broadcasting System v. Sylvania Elec. Products, Inc., 415 F.2d 719, 735, 162 USPQ 577 (1st Cir.1968) cert. denied, 396 U.S. 1061, 164 USPQ 321 (1970).

The claims and analysis

It is clear that there are two affirmative steps in the method, claim 34, as amended recites:

> A method for delivering a nucleic acid molecule into a cell comprising:
>
> (a) *adding* a delivery agent to composition containing the cell in the absence of the nucleic acid molecule, and applying ultrasound energy or electrical energy to the cell, wherein the contacting and applying are performed sequentially or simultaneously; and then
>
> (b) contacting the cell with the nucleic acid molecule, whereby the nucleic acid molecule is delivered into the cell.

Hence the claims require affirmative addition of a delivery agent to the composition that contains the cells. Unger *et al.* does not disclose add a delivery agent to a composition containing a cell.

Second, it is clear from the disclosure of the application, that the medium in which cells are provided **does not** constitute a delivery agent. The specification states

> As used herein, "delivery agent" refers to compositions, conditions or physical treatments to which cells and/or nucleic acids may be exposed in the process of transferring nucleic acids to cells in order to facilitate nucleic acid delivery into cells. Delivery agents include compositions, conditions and physical treatments that enhance contact of nucleic acids with cells and/or increase the permeability of cells to nucleic acids. In all instances, nucleic acids are not directly treated with energy, such as sonoporation.

The specification then describes and provides an exemplary list of delivery agents. For example at page 19, *et seq.*:

> Delivery agents include compositions, conditions and physical treatments that enhance contact of nucleic acid molecules, such as DNA, with cells and/or increase the permeability of cells to nucleic acid molecules, such as DNA. Such agents include, but are not limited to, cationic compounds, peptides, proteins, energy, for example ultrasound energy and electric fields, and cavitation compounds.
>
> Delivery agents for use in the methods provided herein include compositions, conditions or physical treatments to which cells and/or nucleic acid molecules, such as DNA, may be exposed in the process of transferring nucleic acid molecules, such as DNA, to cells in order to facilitate nucleic

> acid molecules, such as DNA, delivery into cells. For example, compounds and chemical compositions, including, but are not limited to, calcium phosphate, DMSO, glycerol, chloroquine, sodium butyrate, polybrene and DEAE-dextran, peptides, proteins, temperature, light, pH, radiation and pressure are all possible delivery agents.

The specification continues and provides lists of exemplary agents. In none of the lists, descriptions or as understood by those of skill in the art, is cell culture medium per se considered a delivery agent.

In addition, addition of serum-free medium is part of the protocol for employing the commercial transfection reagents, such as LIPOFECTAMINE and TRANSFECTAM and CLONFECTIN. The Examiner has provided the manufacturer's instructions for use of the delivery agents, and this includes adding serum free medium for mixing the agent and cells evidencing this. There is nothing in the instructions that discloses that serum free medium is a delivery agent or that it enhances contact. It is widely known to those of skill in the art that serum free medium, an alternative to serum, is defined medium designed to provide nutritive and other essential factors for cell growth and viability in culture. It is not added to cells for delivery of nucleic acids into cells.

In addition, the Examiner's attention is directed, for example, to TABLE 1, Example 7, in the instant application, which sets protocols for various transfection agents (*i.e.*, delivery agents), as well as the text in Example 7 The protocols as described in the application employ serum free medium and/or DMEM as a vehicle in which the cells (or cells and nucleic acid molecules) and delivery agent, such as the cationic lipid compounds, are mixed. This further evidences that these vehicles are not contemplated in the application as 'delivery agents;" addition of such are part of the protocol for addition of one delivery agent. The vehicle is not a delivery agent, and the specification does not support such interpretation.

There is no evidence of record nor is it tenable, that those of ordinary skill in the art would consider that the medium in which reagents are mixed constitutes a delivery agent. The Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well- known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . ..

The statement by the Examiner that the medium in which the cells and delivery agent is mixed constitutes a delivery agent is unsupported. MPEP 2144.03 continues:

> If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious

> character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, there is no evidence that DMEM or serum free medium is a delivery agent, even if one takes the strained and unsupported interpretation of delivery agent relied upon by the Examiner. DMEM and serum-free medium serve as the vehicles in which the delivery agent is added. Knowledge that these vehicles have such properties is clearly not notorious.

It is respectfully submitted that the Examiner has provided no basis to conclude that one of ordinary skill in the art would consider that the protocol for adding a cationic lipid to cells includes addition of two delivery agents: the cationic lipid and the medium in which it is mixed. DMEM or serum free medium are employed to suspend cells for mixing with the agent and the nucleic acid. The specification renders it clear (see, e.g., Table 1 in Example 7), that serum free medium and DMEM and other such media are the vehicles in which the delivery agent is mixed with cells (or cells and nucleic acid molecules) and their use is part of the protocol for adding delivery agents to cells.

As stated above, an anticipatory publication must describe the claimed invention with sufficient clarity and specificity so that one skilled in the relevant art could practice the subject matter of the patent without assistance from the patent claimed to have been anticipated Columbia Broadcasting System v. Sylvania Elec. Products, Inc., 415 F.2d 719, 735, 162 USPQ 577 (1st Cir.1968) cert. denied, 396 U.S. 1061, 164 USPQ 321 (1970). In this instance, it is only by reading Unger *et al.* or any cited reference **with** assistance from the instant application that one of skill in this art could practice the claimed method, which requires use of two delivery agents, not treatment of cells in serum free medium or DMEM, and one delivery agent.

Furthermore, Unger *et al.* discloses delivery of plasmid DNA to cells using ultrasound energy. In the methods of Unger *et al.* plasmid DNA is complexed with liposomes and then ultrasound energy is applied to cells to introduce the plasmid DNA into the cell. Unger *et al.* does not disclose that when energy is applied to the cells, it is applied before contacting the cells with the nucleic acid molecule as required by the instant claims. Further, Unger *et al.* does not disclose delivery of large nucleic acid molecules. Therefore, Unger *et al.* does not disclose all elements as claims, and does not anticipate the rejected claims.

Claims 1-4, 6, 7, 9, 10, 12-14 and 30-32

Claims 1-4, 6, 7, 9, 10, 12-14 and 30-32 are rejected under 35 U.S.C. §102(b) as being anticipated by Marschall *et al.* as evidenced by LIPOFECTAMINe Reagent or TRANSFECTAM Reagent product description. The Examiner urges that Marschall *et al.* discloses introducing large nucleic acid molecules into cells by lipofection and that the product descriptions for LIPOFECTAMINe and TRANSFECTAM includes replacing the serum with serum-free medium. As in the rejection over Unger *et al.*, the Examiner is relying upon an expensive interpretation of delivery agent as including the medium in which the cells are contacted. As argued above, this interpretation is untenable. Accordingly, this rejection respectfully is traversed.

CLAIMS

Independent Claim 1 is directed to a method for introducing a large nucleic acid molecule into a cell by:

(a) contacting a large nucleic acid molecule with a first delivery agent;

(b) contacting adding a second delivery agent to a composition containing the a cell with a delivery agent or applying a delivery agent to the cell, whereby the second delivery agent contacts the cell; and

(c) contacting the cell with the nucleic acid molecule, whereby the nucleic acid molecule is delivered into the cell.

Steps (a) and (b) are performed sequentially in any order , provided that if the delivery agent is energy it is not applied to the nucleic acid molecule and it is not applied to the cell after contacting the cell with the nucleic acid molecule. The first and second delivery agents are different; the first delivery agent increases contact between the nucleic acid molecule and the cell compared to in the absence of the delivery agent; and the second delivery agent enhances permeability of the cell compared to prior to addition of the second delivery agent to the composition. Dependent claims recite additional elements.

The application and rejected claims render it clear that there are two treatments required; this cannot include the cells in their medium as that contradicts the specification and intended elements of the method. As discussed above, the specification clearly states that the methods for delivery include affirmative addition of two agents. For these claims, one agent is intended to increase contact of the nucleic acid molecule with a cell. Exemplary of such agents are cationic lipids, such as LIPOFECTAMINE, not cell culture medium nor other

medium in which such contacting occurs. The second agent is one, such as energy that increases permeability of the cells. Nothing in the specification nor known to those of ordinary skill in the art would lead one of ordinary skill in the art to consider serum free medium to be an agent that increases permeability of cells; rather it is for suspending cells to maintain viability.

Relevant law

Relevant law is provided in previous responses of record and is incorporated herein by reference.

Disclosure of Marschall *et al.* and differences from the instant claims

Marschall *et al.* discloses transfection of YACS into cells using a single cationic amine. Marschall *et al.* does not describe a method in which nucleic acid molecules are contacted with a delivery agent and in which a delivery agent is added to a composition containing cells. As discussed above, addition of serum free medium is part of the protocol for adding a cationic amine to cells. It serves as the medium in which cells are suspended for addition of the cationic amine; there is nothing to indicate that the medium serves as a delivery agent. Further, in the interest of advancing prosecution, claim 1, as amended recites that the first delivery agent increases contact of the nucleic acid with the cell, which cationic amines do; and the second delivery agent increases permeability of the cells.

Not only is serum free medium not a delivery agent, there is no evidence provided that it increases permeability of cells. Serum free medium is employed to suspend cells and to maintain their viability Again, the Examiner is reminded MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well- known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . ..

The statement by the Examiner that the serum free medium in which the cells are suspended increases permeability is not capable of instant and unquestionable demonstration. MPEP 2144.03 continues:

> If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, there is no evidence serum-free medium is used to increase permeability of cells nor evidence that such knowledge is notorious.

Analysis

Marschall *et al.* does not disclose a method for delivering large nucleic acid molecules into cells by contacting nucleic acid molecules with a first delivery agent that increases contact of cells with nucleic acids; and contacting the cells with a second agent that increases their permeability to the nucleic acid molecule. Marschall *et al.* discloses a method in which commercially available delivery agents are used to introduce YACS into cells. As described in the instant application, protocols for use of these commercially available cationic amines include suspending cells in serum free medium; such step does not constitute addition of a second delivery agent, and certainly not one that increases permeability of cells to the nucleic acid molecules. Thus, Marschall *et al.* does not anticipate any of the rejected claims.

Furthermore as stated above with respect to the rejection over Unger *et al.*, an anticipatory publication must describe the claimed invention with sufficient clarity and specificity so that one skilled in the relevant art could practice the subject matter of the patent without assistance from the patent claimed to have been anticipated Columbia Broadcasting System v. Sylvania Elec. Products, Inc., 415 F.2d 719, 735, 162 USPQ 577 (1st Cir.1968) cert. denied, 396 U.S. 1061, 164 USPQ 321 (1970). In this instance, it is only by reading Marschall *et al.* with assistance from the instant application that one of skill in this art could practice the claimed method, which requires treatment of nucleic acid molecules with a contact-enhancing delivery agent, such as a cationic amine, and treatment of the cells with an agent that increases permeability. Marshall *et al.* discloses use of a single delivery agent; not two as required by the instant claims. Nothing in Marschall *et al.* or of record would place one of ordinary skill in the art in possession of a method that requires addition of a second delivery agent to cells to increase their permeability.

THE REJECTION OF CLAIMS 1-10, 12-14, 30-32, 59, 61-64 and 144-147

Claims 1-10, 12-14, 30-32, 59, 61-64 and 144-147 are rejected under 35 U.S.C. §103(a) as being upatentable over the Hadlaczky *et al.* (U.S. Patent No. 6,025,155), which teaches lipid-mediated transfection, in view of Marschall *et al.* as evidenced by LIPOFECTAMIN Reagent or TRANSFECTAM Reagent because Hadlaczky *et al.*, describes introduction of artificial chromosomes, including ACES, into cells using lipid mediated transfer; and Marschall *et al.* teaches use of the commercially available cationic amines, LIPOFECTAMINE and TRANSFECTAM for introduction of YACS into cells. The Examiner concludes that it would have been obvious to have used LIPOFECTAMIN or

TRANSFECTAM for lipid-mediated transfection of large nucleic acid molecules, such as ACES into cells. As with the rejections above, this rejection is premised on the assertion that serum free medium constitutes a delivery agent. This rejection is respectfully traversed.

Relevant law

The relevant law for establishing a *prima facie* case of obviousness is set forth in previous responses and is incorporated herein by reference.

Analysis

The combination of teachings of Hadlaczky *et al.* and Marshall *et al.* does not result in the instantly claims methods

As discussed above, a delivery agent is a something that facilitates introduction of nucleic acid into a cell, such as by enhancing contact of nucleic acids with cells and/or increasing the permeability of cells to nucleic acids compared to its absence. The medium in which the cells are contacted does not meet this definition nor the understanding of those of skill in the art nor other disclosure in the application. The Examiner has provided no evidence to support such assertion.

Hadlaczky *et al.* teaches the use of lipid mediated transfection for introducing large nucleic acid molecules into cells. Marschall *et al.* teaches the use of the cationic amines LIPOFECTAMINE and TRANSFECTAM for introducing YACS into cells. Neither references teaches or suggest a method in which nucleic acid molecules are treated with a first delivery agent that increases contact between the nucleic acid molecules and the cells (compared to in its absence) , and cells are treated with a second, different reagent, that increases permeability of the cells (compared to in its absence). Therefore, the combination of teachings of these references cannot result in the instantly claimed methods, which require these elements. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,

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